Research Article

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Effect of pre-harvest sprouting on physicochemical changes of proteins in wheat

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Abstract

BACKGROUND: High moisture before harvest can cause sprouting of the wheat kernel, which is termed pre-harvest sprouting (PHS). The aim of this study was to examine the variation in physicochemical properties of proteins in PHS-damaged (sprouted) hard red and white spring wheat genotypes. Specifically, protein content, enzyme activity and degradation of proteins were evaluated in sound and PHS-damaged wheat.

RESULTS: Protein contents of sprouted wheat samples were lower than that of non-sprouted samples; however, their differences were not significantly ($P > 0.05$) correlated with sprouting score. Sodium dodecyl sulfate (SDS) buffer extractable proteins (EXP) and unextractable proteins (UNP) were analyzed by high-performance size exclusion chromatography. PHS damage elevated endoprotease activity and consequently increased the degradation of polymeric UNP and free asparagine concentration in wheat samples. Free asparagine is known to be a precursor for formation of carcinogenic acrylamide during high heat treatment, such as baking bread. Free asparagine content had significant correlations ($P < 0.01$) with sprouting score, endoprotease activity and protein degradation.

CONCLUSIONS: Genotypes with higher endoprotease activity tend to exhibit a larger degree of degradation of UNP and higher free asparagine concentration in sprouted wheat samples.

Keywords: spring wheat; pre-harvest sprouting; wheat proteins; free asparagine

INTRODUCTION

Pre-harvest sprouting (PHS) is defined as the premature germination of wheat kernels in the spike under unfavorable environmental conditions. Premature germination causes embryo growth in the wheat kernel while still on the head in the field.[1] PHS has become a main constraint to the production of high-quality cereal end products; for example, bread and sponge cake prepared from sprouted wheat display undesirable quality characteristics.[2] The impact of PHS wheat on the end product depends on enzyme activities present and breakdown of the kernel biochemical components.[3] The physiological changes needed to produce a new plant require energy and nutrients, which is the reason why the sprouted wheat produces enzymes, such as amylases, proteases and lipases, to break down starch, protein and oil, respectively.

The quantity and quality of wheat proteins are critical factors for determining wheat quality and possibly have the most important contribution to bread flour quality. Composition of gluten proteins is commonly believed to be highly correlated with dough strength and baking quality. From a chemical point of view, wheat proteins can be separated into two groups: the low-molecular-weight soluble proteins and the high-molecular-weight insoluble gluten proteins. The soluble groups are made up of albumins, globulins and peptides, and can be dissolved in natural aqueous mediums. The insoluble gluten proteins primarily consist of glutenins and gliadins, which represent 80–85% of the wheat storage proteins. During the mixing procedure of the wheat bread-making process, the intermolecular interaction of the glutenin and gliadin molecules results in gluten formation. Gluten is primarily responsible for the unique viscoelastic and gas-retaining properties of dough made from wheat. High-performance size exclusion chromatography (HPSEC) has been extensively conducted to analyze molecular weight distribution (MWD) of wheat proteins.[4–7] Studies using HPSEC analysis of bread wheat proteins have indicated that polymeric proteins in the sodium dodecyl sulfate (SDS) buffer unextractable protein (UNP) fraction could enhance dough strength, whereas wheat proteins in the SDS buffer extractable protein (EXP) fractions have been shown to be associated with weak dough characteristics.[5,8,9] The polymeric proteins in UNP have a strong effect on dough strength parameters due to the larger associations between high-molecular-weight glutenin subunits.[5]

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Another concern related to the quality of wheat protein is the possible increase in free asparagine due to protein hydrolysis caused by PHS. The elevated proteolytic enzyme activity in sprouted wheat has resulted in increased free asparagine.[10] Acrylamide is known to form when cereal-based foods are heated at high temperatures (>120 °C) during processing, such as bread baking. This is a concern due to potential risks of carcinogenic activity in humans.[11,12] Acrylamide formation in cereal foods is associated with free asparagine, which has been identified as one of the primary precursors.[13,14] Free asparagine has been found to show significant variation among wheat genotypes, and the use of low asparagine cultivars may be one strategy to reduce acrylamide formation in wheat-based foods.[10,15,16]

The extent of PHS damage can range from very minor to severe; it can be measured by percentage of sprouted wheat kernels, starch degradation or other indicators.[2] Elevated endoprotease activity in sprouted wheat will cause degradation of proteins, leading to a reduction in wheat quality and consequently economic losses. Despite the great influence of PHS on proteins, there has been limited research on variations in physicochemical properties of proteins, especially changes of protein MWD, and free asparagine concentration for sprouted samples of hard spring wheat genotypes. The aim of the present work was to determine variations in protein MWD and free asparagine concentration of sprouted samples and their correlations with PHS parameters including endoprotease activity and sprouting score in hard red and hard white spring wheat genotypes.

MATERIALS AND METHODS

Materials

Twenty-four genotypes, including 12 hard red spring (HRS) and 12 hard white spring (HWS) wheat genotypes grown at three locations (Casselton, Carrington, and Prosper, ND) in 2008 were analyzed. Field plot design was a randomized complete block with four replications. 'Hanna' and 'AC Snowbird' have previously demonstrated a high level of seed dormancy. Therefore, Hanna was considered an HRS and AC Snowbird an HWS control for tolerance to PHS. 'Ingot' and 'Lolo', previously exhibiting susceptibility to PHS, were considered PHS-susceptible HRS wheat and HWS wheat types, respectively.[17] In this study, sprouted and non-sprouted wheat samples were analyzed in two replicates. Each replicate contained samples from two replicates of field experiments. Thus physicochemical characterization of protein from a total of 288 samples (24 entries × 2 replicates × 3 locations × 2 – sprouted and non-sprouted) was carried out in the present research. Susceptibility to PHS and score assignments were performed according to the following procedure. Thirty wheat spikes were randomly harvested from each experiment unit at plant physiological maturity and immediately stored at 10 °C to inhibit additional enzyme activity. The spikes were placed in a mist chamber and misted for a period of 48 h. Following the misting, a humidifier was placed in the chamber to maintain high humidity for 3 days at 18–20 °C. The humidifier was set to run for 1 h cycles alternating between on and off. The humidity was at 100% when the humidifier was on.

Sprout score

Wheat samples were evaluated and scored for tolerance to PHS by Mr Mory OP Rugg at the Department of Plant Science, North Dakota State University. These procedures were as follows. At plant physiological maturity, 30 wheat spikes were randomly harvested from each experimental unit. The spikes were immediately stored at 10 °C to inhibit additional α-amylase activity, placed in a mist chamber and misted for a period of 48 h. Following the misting, a humidifier was placed in the chamber for 3 days. Visual observations of the spikes were made to assess the degree of sprouting induced by maintaining high moisture in the misting chamber. Spikes were scored visually 0–9, where 0 represented no visible sprouting and a score of 9 represented very severe sprouting, with average coleoptile length greater than 2 cm.[17]

Protein content

All wheat samples were ground in a cyclone sample mill (Udy, Fort Collins, CO, USA) with a 0.8 mm sieve. Nitrogen content (14%, moisture basis) of each sample was determined by the combustion method (Approved Method 46–30.01),[18] using a LECO FP428 nitrogen analyzer (LECO Corporation, St Joseph, MN, USA).

α-Amylase activity

Wheat samples were dried and ground in a cyclone sample mill (Udy) equipped with a 0.8 mm sieve. Samples of ground wheat (0.5 g) were weighed into test tubes containing stir bars. The test tubes were placed in a stirring/heating block at 60 °C and stirred at medium-high speed. Sodium maleate buffer (5 mL, 100 mmol L^{-1}, pH 6.0) was heated to 60 °C and added to each tube, stirred for 5 min and then an Amylazyme tablet (Megazyme Co. Ltd, Bray, Ireland) was added. The reaction was stopped by adding 6 mL Trizma base (2% w/v, pH 9.5) after exactly 5 min. Subsequently, the sample was left at room temperature for 5 min, then stirred and filtered. The absorbance of the filtrate at 590 nm was measured against the reaction blank and α-amylase activity was calculated by reference to a standard curve.

Endoprotease activity

Endoprotease activity was determined using an azurine-crosslinked casein substrate (Protazyme AK tablet; Megazyme Co. Ltd, Bray, Ireland) according to the procedure reported by Ichinose *et al.*,[19] with some modifications. Extraction buffer (5.0 mL, 100 mmol L^{-1} sodium phosphate buffer, pH 7.0) was stirred with 0.50 g sample in a test tube at room temperature for 30 min. The mixture was centrifuged at 2000 rpm for 10 min. One Protazyme AK tablet was added to 1.0 mL of reaction buffer (100 mmol L^{-1}, pH 6.9, sodium phosphate buffer with 1% SDS, w/v) and stirred for 5 min at 40 °C. Enzyme extract (1.0 mL) was then added to the reaction buffer and stirred at 40 °C for 2 h. Ten milliliters of 2% trisodium phosphate was added to terminate the reaction and filtered through qualitative filter paper into Hach spectrophotometer tubes. The absorbance of the filtrates was measured at 590 nm using a spectrophotometer. One unit of enzyme activity was defined as the change in absorbance per hour per gram of sample at pH 7.0 and 40 °C.[19]

High-performance size exclusion chromatography

EXP and UNP were obtained following the method of Gupta with minor modifications.[20] Extraction buffer was 0.5% SDS and 0.1 mol L^{-1} sodium phosphate buffer (pH 6.9). Flour (10 mg, 14% mb) was suspended in 1 mL extraction buffer and stirred for 5 min at 2000 rpm using a pulsing vortex mixer (Fisher Scientific, Pittsburgh, PA) to solubilize EXP. The mixture was then centrifuged for 15 min at 17 000 × *g* (centrifuge model 5424, Eppendorf, Hamburg, Germany). The supernatant was filtered through a 0.45 µm polyvinylidene membrane (Sun Sri, Rockwood, TN, USA). After filtering, the sample was immediately heated for 2 min at 80 °C

Table 1. Mean, minimum, and maximum protein contents in non-sprouted and sprout damaged wheat samples of hard red and white genotypes harvested at three locations

Location	Class	Grain[a]	Mean[b]	SE[c]	Minimum	Maximum	*F*-value[d]
Carrington	Red	Non-sprouted	14.1***	0.5	13.1	15.1	11.9***
		Sprouted	13.0***	0.6	12.2	14.2	2.6*
		ΔD	1.1**	0.2	0.4	1.3	0.7
	White	Non-sprouted	13.4	0.6	12.6	14.4	15.7***
		Sprouted	12.0	1.0	10.7	13.8	7.8***
		ΔD	1.4	0.7	0.6	2.8	4.9***
Casselton	Red	Non-sprouted	14.5***	0.6	13.4	16.0	2.3*
		Sprouted	12.9**	0.8	11.6	14.6	2.2
		ΔD	1.6**	0.5	1.0	2.6	1.5
	White	Non-sprouted	13.4	1.1	11.9	15.2	6.5***
		Sprouted	12.2	1.0	11.0	14.3	3.2**
		ΔD	1.1	0.6	0.5	2.0	2.0
Prosper	Red	Non-sprouted	14.3***	0.5	13.1	14.8	4.2**
		Sprouted	12.8***	0.5	12.0	13.6	5.6***
		ΔD	1.4	0.3	0.9	1.9	1.2
	White	Non-sprouted	13.4	0.8	12.4	15.1	11.3***
		Sprouted	11.8	0.8	10.9	13.5	14.2***
		ΔD	1.6	0.6	0.5	2.7	4.7***

[a] Difference = non-sprouted − sprouted samples.
[b] Asterisks indicate that difference between red and white mean values is significant at α = *0.05, **0.01 and ***0.001.
[c] SE, standard error values calculated using mean values of genotypes at individual location.
[d] Asterisks indicate that variance for genotype is significant at α = *0.05, **0.01 and ***0.001.

to inactivate endoprotease activity.[21] The UNP was solubilized from the residue after extracting EXP using a sonicator (Sonic Dismembrator 100, Fisher Scientific). The residue was sonicated for 30 s in 1 mL extraction buffer solution at a power setting of 10 W output. The mixture was centrifuged and filtered, and the filtered solution was heated using the same conditions as the EXP. HPSEC was performed using an Agilent 1100 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA). The EXP and UNP were separated by a narrow-bore size exclusion column (BIOSEP SEC S4000, Phenomenex, 300 × 4.5 mm, Torrance, CA, USA) with guard cartridges (BIOSEP SEC S4000).[22,23] Proteins were eluted by 50% acetonitrile in water with 0.1% trifluoroacetic acid at a flow rate of 0.5 mL min^{-1} and detected at 214 nm using a photodiode array detector (1200, Agilent Technologies). The signal was reported in milli-absorbance units (mAU). A wavelength of 650 nm was employed as a reference to compensate for fluctuation caused by changes in baseline absorbance during the HPLC run. The mean of UV absorbance values of first five data-points was used for baseline subtraction before integration. These experiments were duplicated and the mean values were used for data analyses.

HPSEC data collection

Absorbance data from HPSEC of protein extracts was analyzed using MATLAB 2008 (The MathWorks, Natick, MA, USA).[20] Absorbance values were interpolated to 0.002 min intervals by a spline method in MATLAB. Absorbance Area (AA) was calculated by mean absorbance by a time interval of 0.002 min using the interpolated absorbance values. Data collection was performed using the sum of AA for each retention time interval of 0.01 min between 3.6 and 9 min of run time. The AA values for total proteins were mathematically estimated by adding AA values of EXP and UNP.[23] Absorbance area percentage (*A*%) values were calculated for each retention interval of 0.01 min over the total AA.[20] Simple linear correlation coefficients (*r*) were calculated between wheat parameters and *A*% values, and presented as a continuous spectrum over retention time.

Free asparagine analysis

Free amino acids were extracted as described by Mustafa *et al.*,[24] with minor modifications,[25] using the EZ-Faast amino acid kit for gas chromatography and mass spectrometry (Phenomenex, Torrance, CA, USA). The sample (0.2 g, 14%, mb) was mixed with 15 mL ethanol solution (50%, v/v) pre-heated to 50 °C and stirred at a speed of 150 rpm for 20 min at 50 °C in an incubating shaker (MaxQ 4000, Barnstead/Lab-line, Thermo Scientific, Waltham, MA, USA). An aliquot (1.5 mL) was removed and centrifuged for 15 min at 7200 × *g*. After adding 20 nmol norvaline internal standard to an aliquot (200 µL) of the supernatant, the amino acids were separated by solid-phase extraction. The mixture was then subjected to a two-step derivatization at room temperature according to the EZ-Faast procedure. The derivatized amino acids were resolved in isooctane/chloroform (100 µL) and an aliquot of 2 µL was analyzed by gas chromatography (HP 5890 Series II, Hewlett Packard, Palo Alto, CA, USA) equipped with a mass-selective detector (HP 5971, Hewlett Packard)[16] at 280 °C in 1:1 split mode on a Zebron ZB-AAA capillary column (10 m × 0.25 mm; 0.25 µm film thickness). The oven temperature was held at 110 °C for 1 min and then increased by 30 °C min^{-1} to 320 °C after injection. The transfer line and ion source temperature were maintained at 320 and 230 °C, respectively. Carrier gas flow rate was maintained at 0.9 mL min^{-1} throughout the run. A calibration curve was generated using standard solutions provided by Phenomenex (EZ-Faast kit, Phenomenex, Torrance, CA, USA).

Statistical analysis

Statistical analysis was performed using the SAS System for Windows (V. 9.2; SAS Institute, Cary, NC, USA). Bartlett's test

Table 2. Sprout score of sprouted wheat and α-amylase activity and endoprotease activity of sprouted and non-sprouted wheat samples and their differences for hard red and white spring genotypes

		α-Amylase activity (CU[a] g^{-1})			Protease activity (A_{590} g^{-1} h^{-1})		
Genotype	Sprout score	Sprouted	Non-sprouted	ΔD[b]	Sprouted	Non-sprouted	ΔD[b]
HRSW							
Hanna	2.8	1.32	0.11	1.20	2.16	1.40	0.76
Ingot	7.0	2.37	0.10	2.27	2.28	1.50	1.07
Alsen	4.8	1.82	0.09	1.57	2.06	1.76	0.31
Briggs	5.7	2.16	0.11	2.06	2.08	1.43	0.66
Freyr	4.4	1.79	0.09	1.70	2.27	1.42	0.85
Glenn	4.0	1.68	0.08	1.61	2.00	1.51	0.49
Granite	5.3	2.09	0.13	1.96	2.44	1.56	0.88
Kelby	3.4	1.56	0.13	1.43	2.03	1.39	0.64
Norpro	6.0	2.18	0.09	2.09	2.23	1.51	0.72
Reeder	4.4	1.76	0.08	1.68	2.28	1.35	0.94
Steele-ND	5.0	1.93	0.08	1.85	2.15	1.51	0.65
Knudson	5.4	2.12	0.10	2.02	2.27	1.32	0.95
Mean	4.8	1.90	0.10	1.79	2.19	1.47*	0.74
HWSW							
99S0155-14W	2.5	1.36	0.12	1.24	1.92	1.49	0.44
Otis	7.8	2.47	0.11	2.37	2.00	1.50	0.50
AC Snowbird	2.8	1.39	0.08	1.31	2.34	1.26	1.08
AC Vista	5.8	2.13	0.09	2.04	2.56	1.39	0.90
Argent	4.8	1.98	0.16	1.83	2.67	1.48	1.19
CS3100L	6.8	2.33	0.18	2.16	2.48	1.40	1.08
CS3100Q	6.8	2.44	0.14	2.30	2.44	1.32	1.12
Explorer	6.9	2.37	0.16	2.21	2.48	1.46	1.02
Lolo	5.7	2.17	0.12	2.05	2.59	1.41	1.19
MT9420	6.9	2.33	0.14	2.19	2.47	1.41	1.06
NDSW0602	6.3	2.37	0.12	2.24	2.65	1.43	1.22
Pristine	5.0	1.99	0.18	1.81	2.43	1.47	0.97
Mean	5.7	2.11	0.13	1.98	2.40 * **	1.42	0.98 * **
LSD[c]	1.4	0.39	0.05	0.40	0.14	0.20	0.20

[a] CU, Ceralpha units as defined by the Megazyme α-amylase assay kit.
[b] Difference between non-sprouted and sprout-damaged wheat.
[c] LSD, least significant difference ($\alpha = 0.05$) between genotypes.
Asterisks indicate that difference between red and white is significant at $\alpha =$ *0.05, **0.01 and ***0.001, respectively.

was used to analyze the homogeneity of error variance across the three locations. When errors were homogenous, analysis of variance (ANOVA) was performed using the 'Mixed' procedure in SAS assuming location as a random effect and genotype as a fixed effect. The difference between HRSW and HWSW means was analyzed using the 'Contrast' option. The error variances of protein content across the samples of three locations were heterogeneous; thus protein content of the three locations were analyzed separately. The error variance values of asparagine concentration also were not homogeneous and data transformed by natural log were used for ANOVA. The correlation coefficient was calculated for genotype mean values using the 'Corr' procedure in SAS. except for protein content.

RESULTS AND DISCUSSION

Protein content and endoprotease activity

Proteins are considered to be key components of flour, although, they are not the sole factors affecting the end product quality of wheat flour.[26,27] Quantitative and qualitative changes to protein in the wheat endosperm occur during PHS.[28] Mean values of protein content in non-sprouted and sprouted wheat samples harvested at three locations are given in Table 1. For all three locations, the protein content of non-sprouted wheat was higher than sprouted wheat for all cultivars. Perhaps the decrease of protein content of sprouted wheat in the current research was due to the removal of shoot and root tissue during PHS in the wheat cleaning stage.

The HRSW had significantly ($P < 0.001$) higher mean protein content for both non-sprouted and sprouted samples than HWSW for all the three locations (Carrington, Casselton and Prosper). The difference between non-sprouted and sprouted wheat samples (ΔD) in protein content was inconsistent across locations. The protein content of non-sprouted and sprouted samples, and their difference, did not show any significant ($P > 0.05$) correlation with sprouting score for genotypes in the present research (data not shown), indicating that the protein content of non-sprouted and sprouted wheat may not be an effective indicator of the susceptibility of a genotype to PHS.

α-Amylase activity and endoprotease activity as mean values of individual genotypes over three growing locations are presented in Table 2. Although endoprotease existed at very low activity in non-sprouted wheat kernels, wheat genotypes exhibited significantly

Table 3. Correlation coefficients between HPLC absorbance area percentage values and sprouting score and endoprotease activities for hard spring wheat genotypes

		Endoprotease	
HPLC fractions	Sprout score	Sprouted	ΔD[a]
Sprouted			
SDS extractable			
F1	NS	NS	NS
F2	NS	NS	NS
F3	NS	−0.50*	−0.44*
F4	0.74***	0.82***	0.74***
F5	0.81***	0.87***	0.76***
F6	0.72***	0.81***	0.69***
SDS unextractable			
F1	−0.69***	−0.69***	−0.63**
F2	−0.44*	NS	NS
ΔD[a]			
SDS extractable			
F1	NS	0.41*	NS
F2	NS	NS	NS
E3	NS	NS	NS
E4	0.79***	0.91***	0.84***
E5	0.86***	0.91***	0.81***
E6	0.84***	0.89***	0.78***
SDS unextractable			
F1	−0.73***	−0.88***	−0.78***
F2	−0.52**	−0.57**	−0.58**

[a] Difference between non-sprouted and sprout-damaged wheat.
Asterisks indicate that correlation coefficient is significant at
* $P < 0.05$,
** $P < 0.01$ and
*** $P < 0.001$, respectively; NS, not significant ($P > 0.05$).

different levels of endoprotease activity (Table 2). These results were similar to those of Huang and Varrianomarston,[29] who reported that significant differences occurred among Kansas hard white wheat genotypes for α-amylase activity. Sprouted samples had a larger variation in endoprotease activity among genotypes than non-sprouted samples. The ΔD values for endoprotease activity also varied significantly among genotypes. Hanna had the lowest endoprotease activity (2.00 A_{590} g $^{-1}$ h^{-1}) for sprouted HRSW and the lowest sprout score (2.8) among the HRSW genotypes. Ingot had the highest sprouting score (7.0) and the highest endoprotease activity (2.44 A_{590} g^{-1} h^{-1}) among the HRSW genotypes. HRSW genotypes had a significantly ($P < 0.001$) lower mean of endoprotease activity for sprouted samples and ΔD values than HWSW genotypes. 99S0155-14W, a HWSW genotype, had lower endoprotease activity (1.92 A_{590} g^{-1} h^{-1}) and sprout score (2.5) for sprouted samples than that of all other genotypes. These results indicate that genotypes with low susceptibility to PHS can be segregated on the basis of endoprotease activity for HWSW genotypes.

Huang and Varrianomarston[29] reported that α-amylase activity was positively correlated with degree of PHS damage and this was mainly due to the genotypic differences. Correlations between sprouting score and endoprotease activities were determined for genotypes. Significant and positive correlation occurred between sprouting score and endoprotease activity ($r = 0.88$, $P < 0.001$) in sprouted wheat samples. Sprouting score also had significant correlations with the ΔD values of endoprotease activity ($r = 0.86$, $P < 0.001$). These results indicate that genotypes with greater sprouting score exhibited higher endoprotease activities, and the protein of those genotypes would be degraded more extensively, which is in agreement with the finding of Huang and Varrianomarston.[29] There was a significant and positive correlation between α-amylase and endoprotease activity ($r = 0.88$, $P < 0.001$) in sprouted wheat, further indicating that PHS significantly increased both α-amylase and endoprotease activities. However, the correlation between endoprotease activity and sprouting score ($r = 0.88$, $P < 0.001$) was relatively lower than the correlation between α-amylase activity and sprouting score ($r = 0.98$, $P < 0.001$) in sprouted wheat samples.

Protein molecular weight distribution

HPSEC profiles were divided into six fractions (Fig. 1): F1 (3.6–5.0 min), F2 (5.0–6.0 min), F3 (6.0–6.9 min), F4 (6.9–7.6 min), F5 (7.6–8.2 min) and F6 (8.2–9.0 min).[23,30,31] Larroque *et al.*[32] showed electrophoresis patterns of protein fractions separated by HPSEC. Primary components of each fraction were high-molecular-weight polymeric protein for F1; low-molecular-weight polymeric proteins for F2; gliadins for F3; albumin and globulins for F4; and hydrolyzed polymeric protein for F5 and F6.[30–32]

Typically, the analysis of wheat proteins using HPSEC produces chromatograms that exhibit six main protein fractions based on their molecular weight (F1–F6). HPSEC profiles of EXP and UNP obtained from non-sprouted and PHS damaged (sprouted) wheat samples are shown in Fig. 1. EXP in sprouted samples showed larger absorbance values for F4, F5 and F6 fractions than that of non-sprouted wheat. Absorbance values of F1 and F2 of UNP in non-sprouted samples was larger than that of sprouted samples at the earlier retention time. This indicated that non-sprouted samples had more polymeric UNP than sprouted samples, and some portion of UNP was shifted to EXP. The shift from UNP to EXP was most likely a result of increased endoprotease activity hydrolyzing the UNP as a result of PHS damage. This result was in agreement with the findings of Hwang and Bushuk,[28] who reported a marked decrease in the amount of insoluble residue protein in sprouted wheat samples.

Hard spring wheat genotypes had significant variation for HPSEC *A*% values of non-sprouted and sprouted samples and their ΔD values (Fig. 1). Significant variation was also observed for the HPSEC *A*% values of F1 and F2 in UNP among genotypes. The high-molecular-weight polymeric protein fraction (F1) in UNP has a positive effect on dough strength parameters due to the associations with high-molecular-weight glutenin subunits.[5,20] Glenn and NDSW0602 had a greater proportion of UNP polymeric proteins in non-sprouted samples, indicating that these cultivars could have stronger gluten than other genotypes when no PHS occurred. Sprouted samples showed a larger variation for HPSEC *A*% values than that of non-sprouted sample for all genotypes. For sprouted samples, Hanna (high tolerance to PHS) had a greater HPSEC *A*% value for F1 of UNP and lower values for F4, F5 and F6 of EXP than other genotypes. To show the detrimental effect of PHS on MWD of wheat proteins, HPSEC profiles of total proteins extracted from sprouted samples of Hanna and Ingot were compared in Fig. 2, since they had the lowest and highest endoprotease activity among HRSW genotypes, respectively (Table 2). When the retention time reached 6.9 min, Ingot showed higher absorbance for EXP than Hanna, which was also observed between non-sprouted and sprouted samples in Fig. 1. Individual genotypes

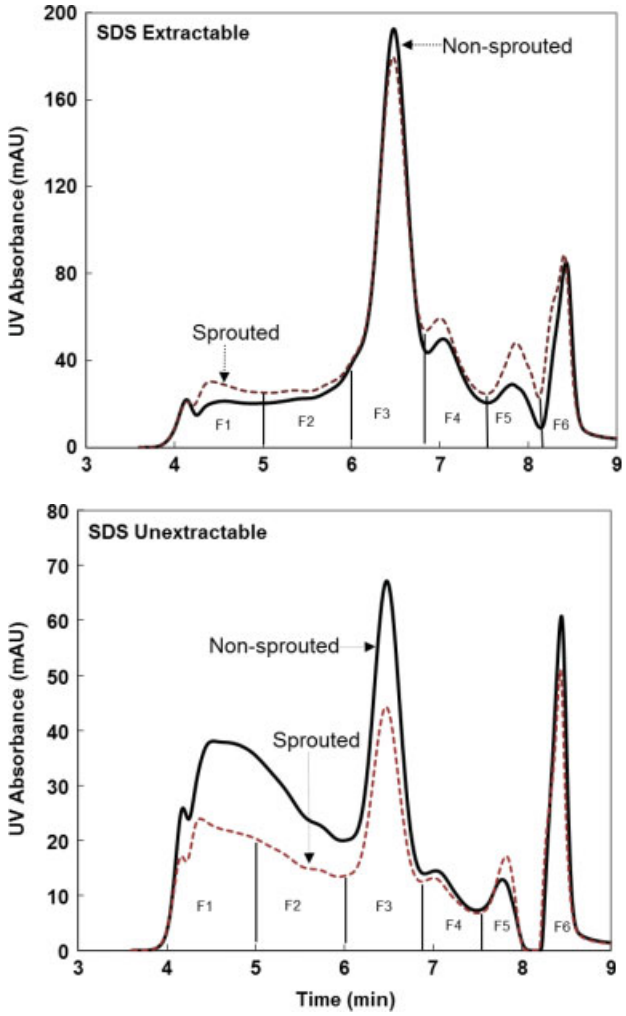

Figure 1. Typical high-performance size exclusion chromatography profiles of SDS buffer extractable and unextractable proteins from sound and sprouted wheat samples.

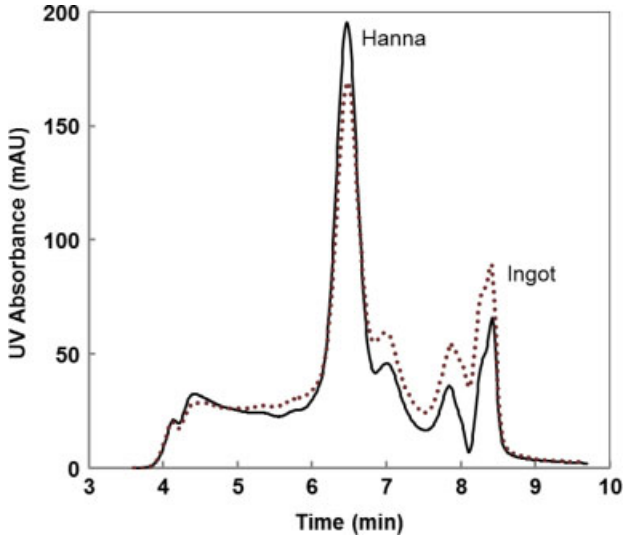

Figure 2. HPSEC profiles of total proteins extracted from sprouted samples of Hanna and Ingot.

exhibited a significantly different degree of protein degradation by PHS.

Means of HRSW and HWSW genotypes were significantly ($P < 0.001$) different for *A*% values of F4, F5 and F6 in EXP for sprouted and non-sprouted samples and their ΔD values (Figs 1 and 2). While no significant ($P > 0.05$) difference in *A*% values of UNP F1 and F2 was detected for non-sprouted samples between mean values of HRSW and HWSW genotypes, HWSW genotypes had significantly ($P < 0.001$) greater mean values for *A*% of UNP F1 in sprouted samples and ΔD. HWSW genotypes could exhibit significantly higher susceptibility to the degradation of high-molecular-weight polymeric proteins in UNP by PHS when compared to HRSW genotypes. However, segregation of genotypes that have tolerance to protein degradation by PHS seems to be possible in HWSW, because two HWSW genotypes – 99S0155-14W and AC Snowbird – showed lower ΔD values for F4, F5 and F6 of EXP and higher values for F1 and F2 of UNP than mean of HRSW genotypes in this research.

Correlation coefficients between HPSEC *A*% values and sprouting score and endoprotease activity are given in Table 3. Sprouting score and endoprotease activities had significant ($P < 0.001$) and positive correlations with HPSEC *A*% and ΔD values of F4, F5 and F6 in EXP, and negative correlations with those of F1 in UNP for sprouted samples. Endoprotease activity is generally related to increased α-amylase activity during PHS, and excessive endoprotease activity has a negative effect on dough handling and baking properties.[19,29] Endoprotease activity of sprouted samples had greater correlations with HPSEC parameters than sprouting score in this research. The ΔD values of endoprotease activity also had a similar pattern of correlation with HPSEC *A*% values. Beresh[33] and Redman[34] reported that the rapid softening of gluten washed from flour milled from grist that included a small amount of sprouted wheat was due to proteolytic hydrolysis of the gluten proteins. The results in the current research indicate that hard spring wheat genotypes exhibited significant variation in endoprotease activity in sprouted samples and it consequently affected the degree of protein degradation. Simple linear correlation coefficients were shown more specifically between endoprotease activity and HPSEC *A*% values as a spectrum over profiles of EXP and UNP (Fig. 3). At the early retention time (3.6–6.0 min), endoprotease activity of sprouted wheat and HPSEC absorbance *A*% of UNP had significant and negative correlations ($r < -0.70$); at the later retention time (6.9–8.2 min), endoprotease activity had significant and positive correlations with EXP ($r > 0.70$). The genotypes with greater endoprotease activity can undergo more hydrolysis of proteins and shift to low-molecular-weight EXP that are undesirable for bread-making quality.

Free asparagine concentration

Free asparagine concentration in sprouted samples is given together with the natural logarithm of the number in Table 4. Mean asparagine concentration of HRSW was significantly ($P < 0.05$) lower than that of HWSW genotypes. Despite that, the segregation of a low-asparagine genotype appeared to be possible for HWSW because 99S0155-14W had the lowest asparagine concentration 608.9 ($\mu g\, g^{-1}$, dry basis) among all the hard spring wheat genotypes analyzed in this experiment. A similar pattern regarding to response of HRSW and HWSW genotypes to PHS was also observed for endoprotease activity (Table 2) and protein HPSEC data in the current research.

Variation in endoprotease activity in hard spring wheat genotypes appeared to affect asparagine concentration in

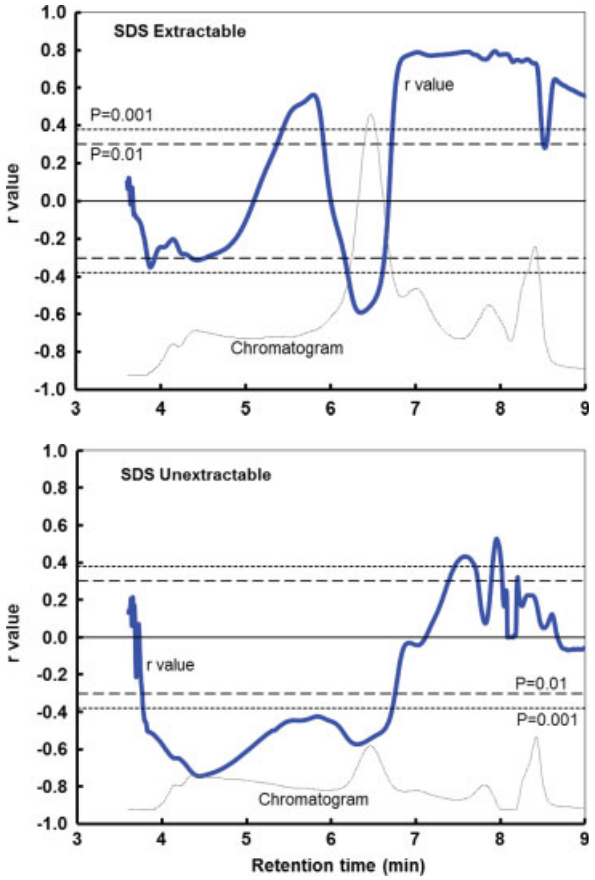

Figure 3. Spectrum of simple linear correlation coefficients (*r*) between endoprotease activity and HPSEC absorbance area percentage values of SDS buffer extractable and unextractable proteins of sprouted samples.

sprouted samples. Hanna and 99S0155-14W had low endoprotease activity (Table 2) and were observed also to have lower asparagine concentration than other HRSW and HWSW genotypes, respectively. Free asparagine concentration had significant ($P < 0.01$) correlations with endoprotease activity, HPSEC *A*% values of F4, F5 and F6 of EXP and F1 of UNP, and their ΔD values for sprouted samples (Table 5). These results indicate that for individual genotypes in which PHS resulted in elevated endoprotease activity there was more hydrolysis of proteins and subsequently higher asparagine concentration in sprouted samples. Freyr, an HRSW genotype, contained high free asparagine concentration in spite of low endoprotease activity of sprouted samples and ΔD value (Table 2). This might be due to the high endoprotease activity in non-sprouted kernel of Freyr that might act to increase asparagine concentration before PHS damage.

CONCLUSIONS

Overall, PHS damage caused significant changes to the protein present in the sprouted wheat. Variation was observed among hard spring wheat genotypes for elevation of endoprotease activity, protein degradation and free asparagine concentration when subjected to PHS damage. Endoprotease activity significantly affected the variations in protein degradation and free asparagine concentration in sprouted samples of hard spring wheat genotypes. Genotypes with the higher endoprotease activity tended to exhibit a larger degree of degradation of UNP that are primarily responsible for gluten strength. PHS-damaged wheat had higher levels of free asparagine, which is undesirable since it is a precursor to acrylamide formation in baked products. HWSW genotypes were generally more susceptible to PHS damage when compared to HRSW genotypes. However, it seems possible to segregate HWSW genotypes that are tolerant to PHS since genotypes such as 99S0155-14W were identified to have great tolerance with low elevation of endoprotease activity, protein degradation and asparagine concentration when subjected to PHS. Endoprotease activity and protein HPSEC data, specifically *A*% value of F5 of EXP, were identified to be good indexes to evaluate the degradation of proteins by PHS.

Table 4. Free asparagine concentration in sprouted samples of hard spring wheat genotypes

Genotype	Asparagine ($\mu g\ g^{-1}$, db)	Asparagine (Log_e)
Red		
Alsen	925.1	6.83
Briggs	1079.0	6.98
Freyr	1173.1	7.07
Glenn	785.6	6.67
Granite	876.2	6.78
Hanna	618.7	6.43
Ingot	1078.6	6.98
Kelby	676.5	6.52
Norpro	1376.0	7.23
Reeder	775.8	6.65
Steele-ND	1355.7	7.21
Knudson	1228.6	7.11
Mean	995.7	6.87
White		
99S0155-14W	608.9	6.41
AC Snowbird	911.6	6.82
AC Vista	885.8	6.79
Argent	1233.2	7.12
CS3100L	1278.8	7.15
CS3100Q	870.5	6.77
Explorer	1376.0	7.23
Lolo	1319.6	7.19
MT9420	1567.9	7.36
NDSW0602	957.1	6.86
Otis	1369.8	7.22
Pristine	1087.2	6.99
Mean	1122.2	6.99*
LSD[a]	—	0.40

[a] LSD, least significant difference ($\alpha = 0.05$) between genotypes. Asterisk indicates that difference between red and white is significant at $^*\alpha = 0.05$.

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Table 5. Correlation coefficients between free asparagine concentration in sprouted wheat samples and sprout score, enzyme activities, and HPLC protein fractions for hard spring genotypes

Variable	Correlation coefficient
Sprouting score	0.68***
Endoprotease: sprouted	0.69***
ΔD[a]	0.60**
Protein content: non-sprouted	NS
sprouted	NS
ΔD[a]	NS
HPLC fraction	
Sprouted: SDS extractable F1	NS
F2	NS
F3	NS
F4	0.61**
F5	0.67***
F6	0.68 ∗ **
Sprouted: SDS unextractable F1	−0.71***
F2	−0.53**
ΔD[a]: SDS-extractable F1	NS
F2	NS
F3	NS
F4	−0.70***
F5	−0.63**
F6	−0.71***
ΔD[a]: SDS-unextractable F1	0.64****
F2	NS

[a] ΔD, difference between non-sprouted and sprouted wheat.
Asterisks indicate that correlation coefficient is significant at
** $P < 0.01$ and
*** $P < 0.001$; NS, not significant ($P > 0.05$).

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